

UNITED STATES
ATOMIC ENERGY COMMISSION
OAK RIDGE
TENNESSEE

DETERMINATION OF SMALL AMOUNTS OF BERYLLIUM
BY FLUORESCENCE MEASUREMENT

by

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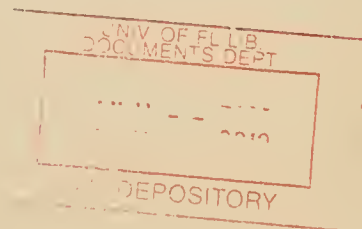
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Date of Manuscript: February 1947

Document Declassified: May 8, 1947

This document consists of 13 pages.





ABSTRACT

The fluorescence of alkaline beryllium solutions with quinizarin has been studied in detail. pH, dye concentration and many common ions have been shown to influence the fluorescence of the complex. From these studies a procedure was developed for the determination of beryllium in amounts of 1-10 micrograms. There is good reason to believe that the method can be extended to determine even smaller amounts of beryllium.

DETERMINATION OF SMALL AMOUNTS OF BERYLLIUM BY FLUORESCENCE MEASUREMENT

I. INTRODUCTION

White and Lowe (1) described the fluorescence of alkaline beryllium solutions with 1-amino-4-hydroxyanthraquinone. Fairhall and his co-workers (2) found that this fluorescence was proportional to the beryllium concentration in the range of 0.05 to 10 micrograms. These investigators studied the fluorescence in ultra-violet light by visual comparison. It is further stated (2) that 1,4-dihydroxyanthraquinone (quinizarin) produced the fluorescence as well as the amino compound.

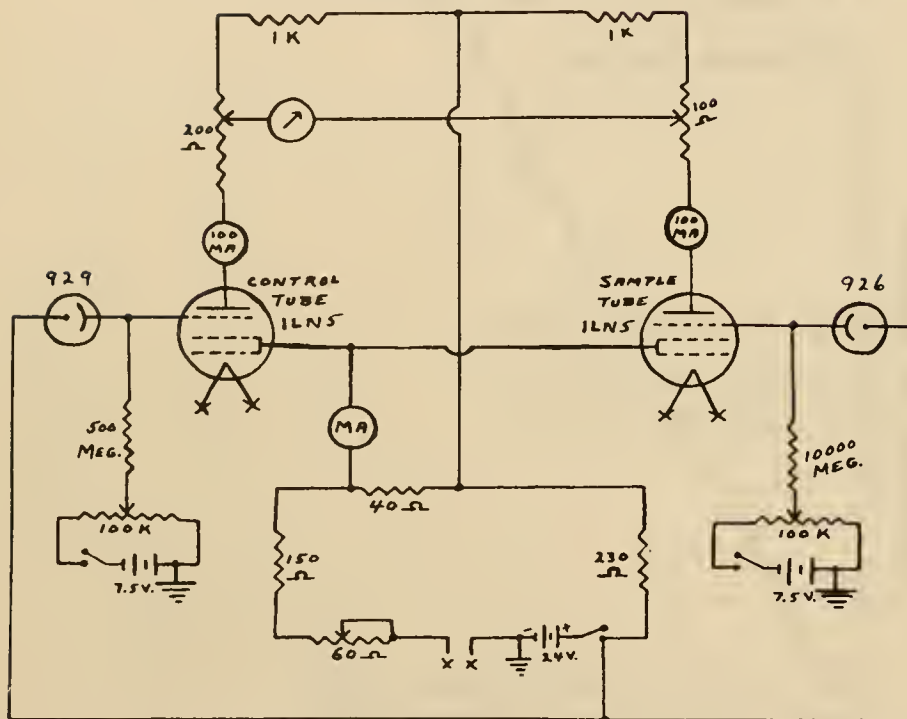
Our attempts to reproduce the method of Fairhall (2) led to anomalous results; therefore studies of the effects of pH, dye concentration, time of standing, and interfering ions were undertaken. Quinizarin was employed in these studies. A procedure based on fluorescence measurement has been developed for determining beryllium in the range of 1 to 10 micrograms per 20 ml, with a standard error of about 10%.

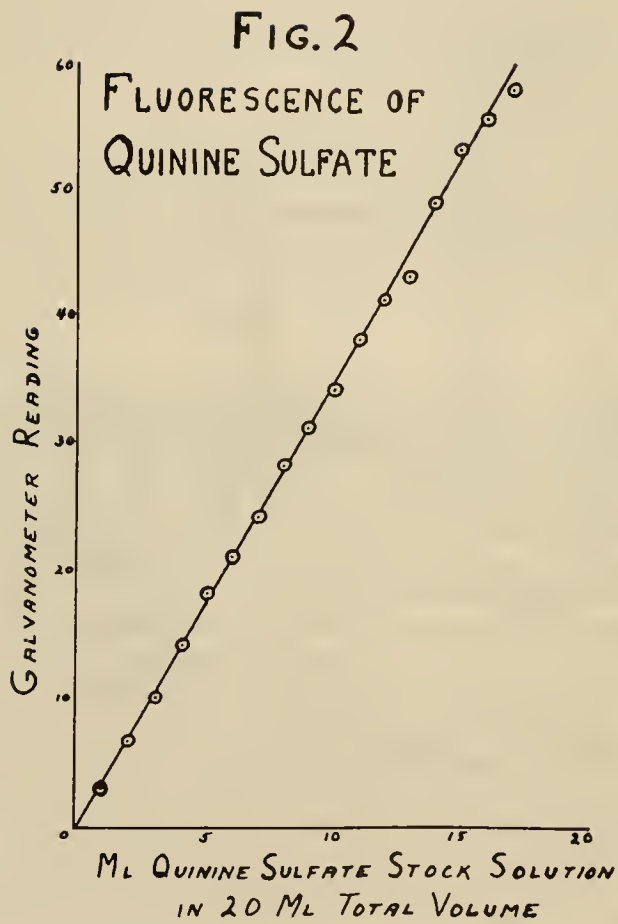
II. EXPERIMENTAL

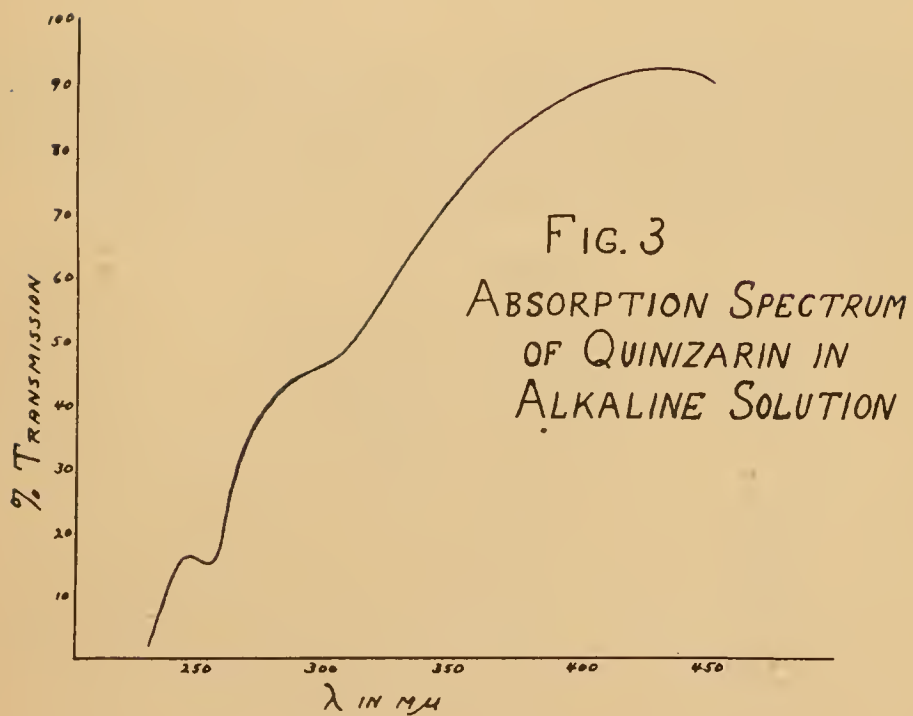
Instrument-Fluorescence intensities were measured by means of the fluorophotometer (Fig. 1) designed and built in this laboratory. This instrument employed two phototubes (and amplifiers), one (the control tube) receiving light directly from the ultra-violet lamp, the other being activated by fluorescence of the sample. A balanced circuit was thus obtained, although null-point measurement with a slide-wire was abandoned in favor of direct galvanometer readings. The ultra-violet source was a General Electric Type H4 mercury discharge lamp. Heat resistant Corning ultra-violet filters (no. 5874) were employed to reduce visible light produced by the lamp; Corning filters (no. 3486) were interposed between the sample chamber and the phototube to absorb ultra-violet light and to transmit the orange-red fluorescent light. The lamp was operated with a constant-voltage transformer; this, together with the balanced circuit mentioned above and shown in the diagram, rendered the instrument insensitive to changes in line voltage which are frequently encountered. The fluorophotometer circuit was operated with four commercial-type, lead storage batteries arranged in series. Cuvettes made of high-silica glass (Klett Mfg. Co.) were used to hold the solutions. The instrument was checked for stability and linearity of response by measuring the fluorescence of quinine sulfate solutions of various strengths (Fig. 2), Corning filters (no. 4303, 3385, and 3389) being used in place of no. 3496 to pass the bluish fluorescence of quinine sulfate.

The adsorption spectrum of quinizarin in alkaline solution was determined, using a Beckmann photoelectric spectrophotometer. From Fig. 3 it is seen that the solution does not strongly absorb light of the principle wave-length of the lamp output (3650 Angstroms). This is important, since penetration of the ultra-violet light is necessary to activate fluorescence throughout the solution when beryllium is present. It was found, however, that addition of beryllium did not significantly alter the absorption spectrum of the solution. This may be disadvantageous, since absorption of the activating light is a prerequisite for fluorescence.

FIG. 1
FLUOROPHOTOMETER
CIRCUIT







Reagents-1,4-dihydroxyanthraquinone (quinizarin), obtained as the technical grade reagent from Eastman Kodak Co., was sublimed, and recrystallized from ethyl alcohol, yielding an orange-red powder having a melting point of 194-195°C. It is moderately soluble in alcohol, forming an orange solution, and is very soluble in alkalis, giving rise to a deep purple solution. In the studies reported below, a solution of 0.3 mg per ml 95% ethyl alcohol was used. Solution was effected by gentle warming over a steam-bath.

Standard beryllium solutions were prepared by dissolving 1 g of the metal in dilute HCl, diluting to a liter, and using 1 ml of this solution to prepare a liter containing 1 microgram beryllium per ml.

Diethylamine (Eastman Kodak Co.) was redistilled (B. P. ca. 55°C) and dissolved in water to give a 1M solution.

Effect of pH- A series of solutions containing 5 micrograms beryllium and 0.2 ml dye solution in a total volume of 20 ml was prepared, using various amounts of NaOH to vary the pH from 7.9 to 13.5. It is important to add the alkali before the addition of the dye; otherwise, a difficultly-soluble precipitate of dye forms. Blanks were prepared for each solution. The pH of each solution was determined by means of a Beckmann pH meter. It was found that maximal fluorescence occurs at pH 11.3 - 11.4 (Table I, Fig. 4).

Table I

EFFECT OF pH ON FLUORESCENCE

<u>pH</u>	<u>Galvanometer Reading</u> <u>(Sample - Blank)</u>
7.90	-11
9.60	-4
10.28	6
10.65	18
10.90	32
11.10	49
11.30	62
11.40	61
11.60	48
11.70	34
11.80	24
13.50	2

Because the pH was shown to be critical, the solutions were buffered in all further studies. It was established by titration studies, that a combination of diethylamine and its hydrochloride was a useful buffer at the pH 11.4. A solution of 2 ml of 1M diethylamine plus 4 ml 0.1N HCl diluted to 20 ml had a pH of about 11.4 which was unchanged by addition of small amounts of beryllium and dye. By comparison of solutions thus buffered with a solution adjusted to the same pH with NaOH, it was established that the buffer does not interfere with the beryllium fluorescence.

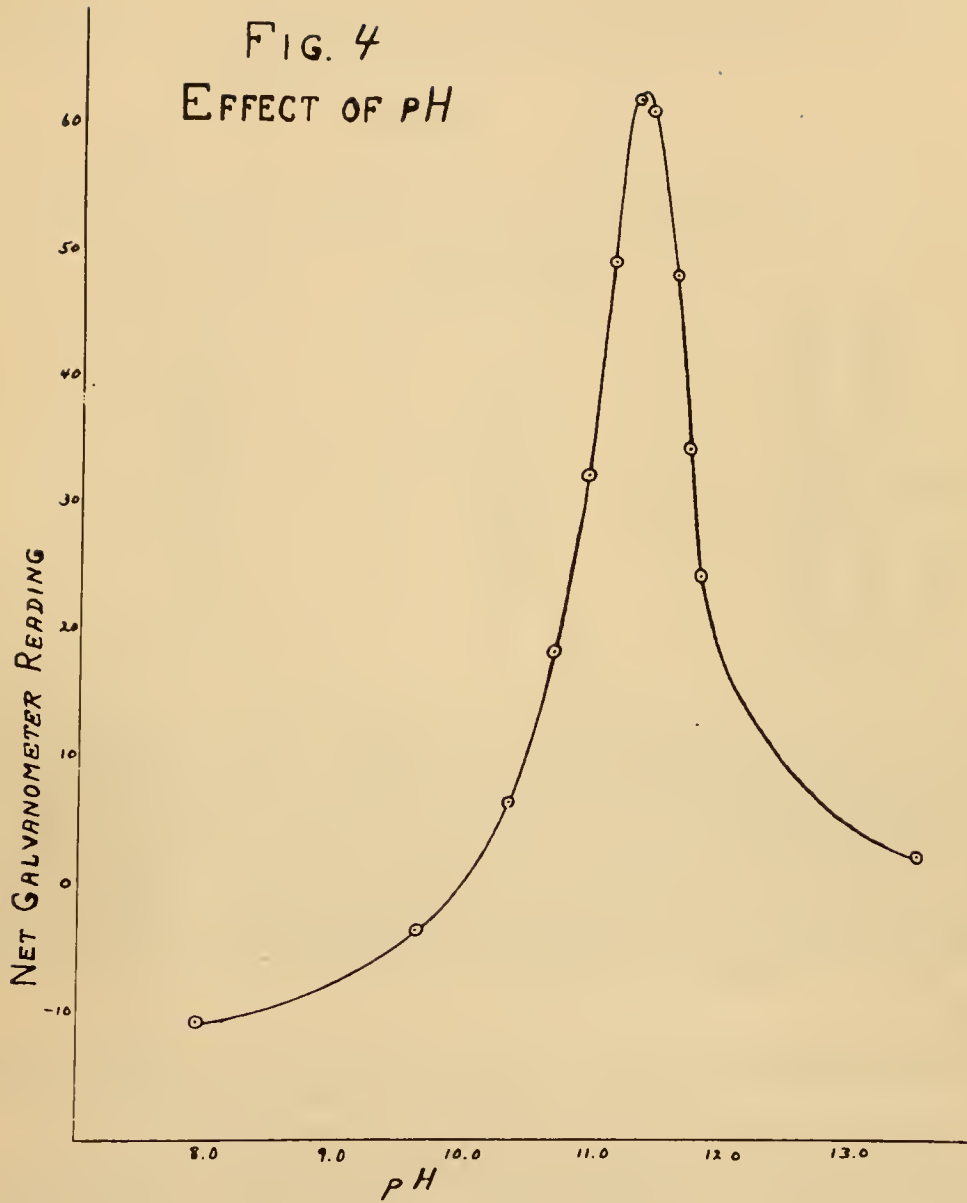
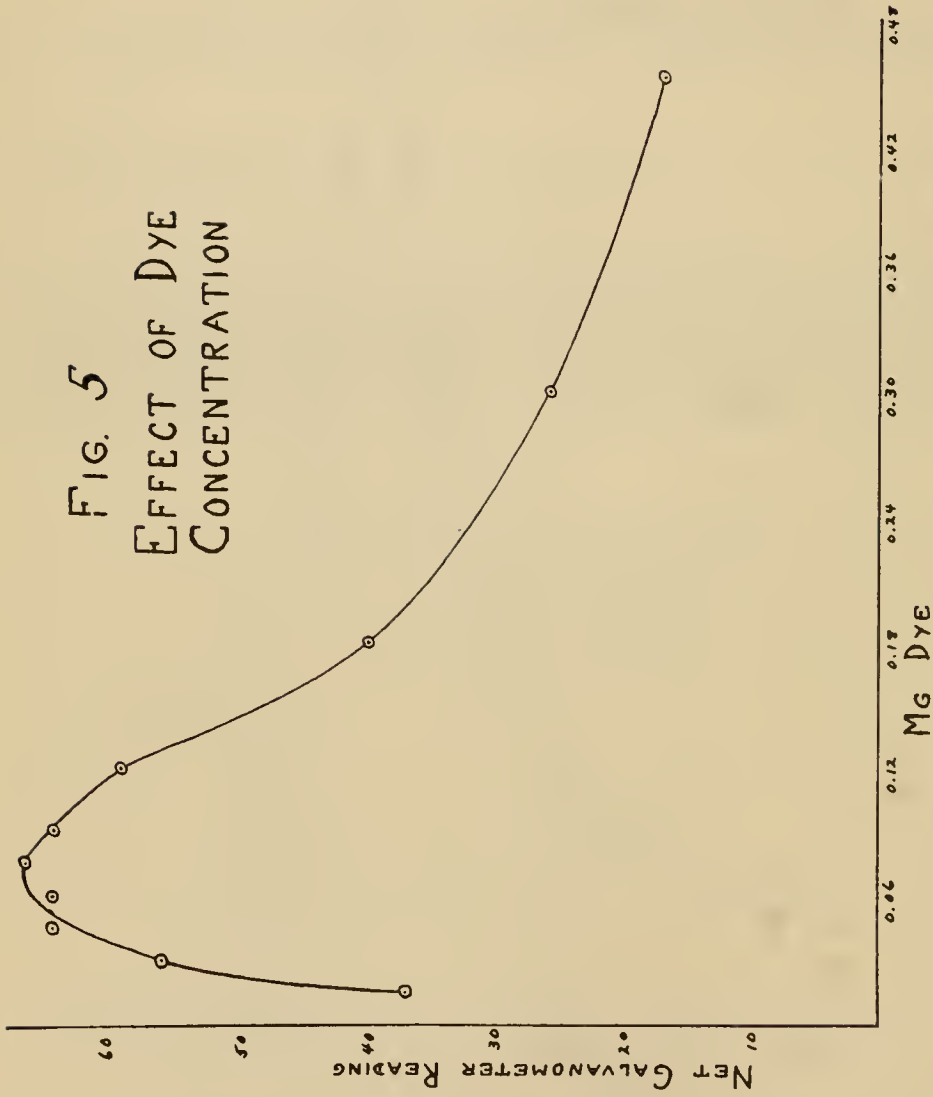


FIG. 5
EFFECT OF DYE
CONCENTRATION



Effect of Dye Concentration-Solutions of constant pH and beryllium concentration, with varying amounts of dye, were prepared. Table II and Fig. 5 show that the dye concentration is critical. This was repeated for several beryllium levels, and it was found that for each beryllium concentration there was an optimal amount of dye which gave the maximal fluorescence. Fig. 6 shows a plot of beryllium concentration (in micrograms per 20 ml) vs. the amount of dye in mg. giving maximal fluorescence).

Table II

EFFECT OF DYE CONCENTRATION ON FLUORESCENCE

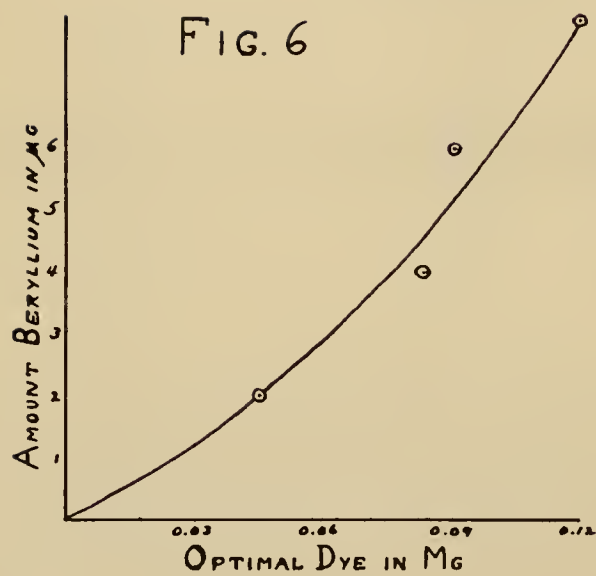
<u>Mg Dye</u>	<u>Net Galv.</u> <u>(Sample-blank)</u>
0.015	37
0.030	56
0.045	64
0.060	64
0.075	66
0.090	64
0.120	59
0.180	40
0.300	26
0.450	17

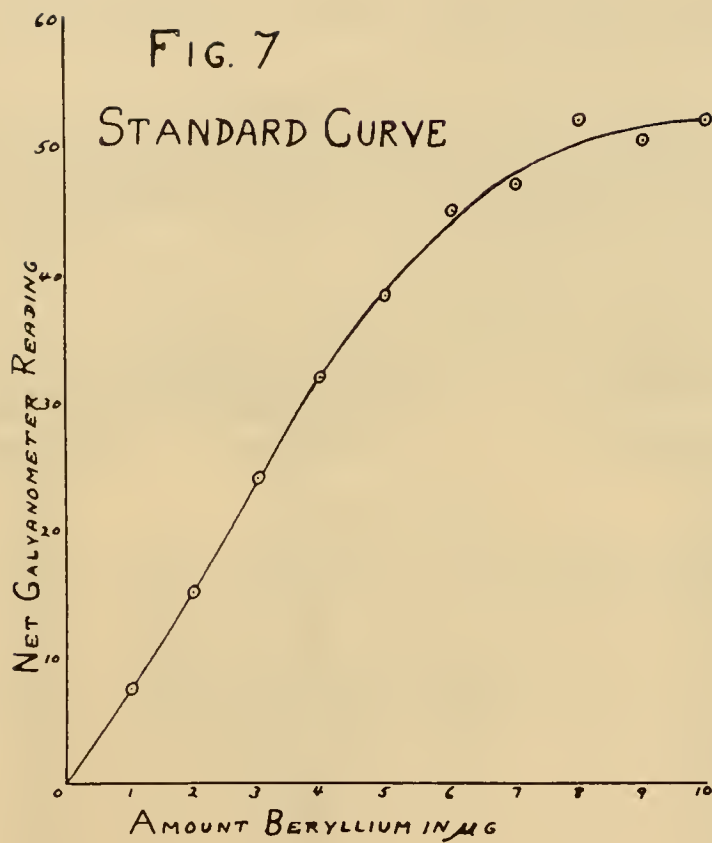
In preliminary test tube experiments, it was noted that ether extracted dye from the aqueous layer in alkaline solutions containing no beryllium, while if beryllium was present, dye remained in the aqueous layer. Therefore an attempt was made to obtain automatically the optimal dye concentration by adding excess dye to several solutions containing from 1 to 10 micrograms of beryllium and extracting the excess with ether, with the idea in mind that the beryllium might hold in the aqueous layer the appropriate amount of dye. This proved not to be the case. Although excess dye was extracted preferentially, as indicated by increased fluorescence of solutions low in beryllium, some essential dye was also removed, since solutions of greater beryllium concentration fluoresced less strongly after extraction.

Effect of Time Standing-No measurable changes in fluorescence intensity occurred in solutions standing for as long as an hour-and-a-half.

Interfering Substances-Fairhall (2) mentioned the interference of calcium and magnesium in determining beryllium in bone samples by the fluorescence method; these precipitated as phosphates from alkaline solution. Beryllium recoveries from such solutions were corrected by factors obtained with bone solutions having known beryllium content.

In the present investigation, various common ions were added in ratios to beryllium of 10:1, 100:1, and 1000:1. Na^+ , PO_4^{3-} , F^- , HCO_3^- , Cl^- , NO_3^- , and SO_4^{2-} were found not to interfere with the fluorescence in the above ratios. Ca^{++} , Mg^{++} , Fe^{++} , Cu^{++} , and Mn^{++} definitely interfered in ratios of 10:1, fluorescence measurements being low by as much as 30-50%.





In larger amounts, the hydroxides of these metals precipitated, making fluorescence measurements impossible.

Procedure for Determining Beryllium in Pure Solution-The total volume selected as convenient for fluorimeter employed was 20 ml. Unknown saturations containing from 1 to 10 micrograms of beryllium were treated as follows: 2 ml 1M diethylamine were added, followed by 0.3 ml dye solution and 4 ml 0.1N HCl; the solutions were then diluted to 20 ml and the fluorescence measured. Beryllium content was read from a standard curve obtained by treating known beryllium solutions in the same manner. Larger amounts of beryllium could be determined by dilutions to give samples within the concentration range required. A typical standard curve is shown in Figure 7.

Results-Two groups of "unknowns", one with 24 samples, the other with 20 samples, were analyzed, using the procedure outlined above. Comparisons of the analytical recovery with the true values are shown in Table III. The standard deviation (sigma) was computed according to the formula: $\sigma \text{ in } \% = \frac{\Sigma(d^2)}{N}$ where d is the percentage deviation for each sample and N is the total number of samples.

TABLE III

<u>Group 1</u>		<u>Group 2</u>	
<u>Analyst's Report Actual Be Content</u>		<u>Analyst's Report Actual Be Content</u>	
micrograms	micrograms	micrograms	micrograms
3.2	3.4	5.6	5.0
3.5	4.0	3.4	3.0
7.9	6.9	10.0	10.0
4.3	5.1	1.0	1.0
4.3	4.6	8.3	6.2
5.3	6.0	2.4	2.0
9.0	9.0	4.5	4.1
10.0	7.0	3.2	3.0
1.9	2.0	7.8	7.0
8.6	8.0	6.0	5.0
3.6	4.0	4.3	4.4
5.2	5.4	10.0	8.0
4.8	5.5	3.7	3.5
1.3	1.0	10.0	9.0
3.4	2.5	5.4	5.0
4.3	4.5	7.4	6.0
2.9	3.0	10.0	7.0
6.8	8.5	5.7	5.5
3.5	3.5	4.0	4.0
5.5	6.0	6.2	6.0
1.5	1.5		
5.3	5.6		
4.0	5.0		
8.2	10.0		
$\sigma \text{ in } \% = 17$		$\sigma \text{ in } \% = 14$	

III. DISCUSSION

The accuracy to be expected by application of the method to pure beryllium samples can be seen from Table III above. The errors are larger than might be desired, but the method may be useful, for the determination of small amounts of beryllium in the absence of more accurate methods. Interferences of common cations as described above indicate that isolation of beryllium will be necessary for any general application of the method, as, for example, in analysis of biological samples, minerals, and metals.

In the work described here, the sensitivity of the instrument was reduced to obtain greater stability for performing fundamental studies of the method. The fact that the blank does not appreciably fluoresce suggests the possibility of applying the method to smaller amounts of beryllium (0.1 microgram and possibly 0.05 microgram per 20 ml.)

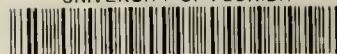
It will be seen from Fig. 7 that the standard curve is not linear throughout the range 1 to 10 micrograms. This was to be expected from the studies of the effect of dye concentration on the fluorescence. If a constant amount of dye, optimum for the center of the range, be added to solutions containing from 1 to 10 micrograms, solutions in the upper part of the range will not contain sufficient dye to activate maximal fluorescence, while in the lower samples, excess dye, which perhaps absorbs part of the fluorescent light, causes lower readings than would otherwise be expected. Thus, workers wishing to apply the method to amounts of beryllium not considered in this report should use care in selecting the amount of dye.

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